

EFFECT OF SOFT X-RAYS ON AUREOMYCIN

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In view of the consideration which has been given to the use of aureomycin in cases of radiation injury produced by atomic bombs (Cronkite, 1951), it is of interest to investigate the effects radiations might have on this substance. Such studies have been made on other compounds (O'Meara, 1952). This paper considers the effects of irradiation of aureomycin with soft x-rays of high intensity.

MATERIALS AND METHODS

Aureomycin hydrochloride (Lederle) was used in three states: crystalline, aqueous solution, and frozen aqueous solution. Twenty-five mg of the solid or 2 ml of a 100 $\mu\text{g}/\text{ml}$ solution were placed in a Lucite (DuPont) cup 17 mm in diameter and 6 mm deep (with the solution protruding from the cup with a convex meniscus). When frozen samples were desired, this cup containing the solution was placed in a recess in the top of a brass rod whose lower end was immersed in a solid CO_2 -acetone bath. For such samples, this freezing arrangement was maintained during irradiations.

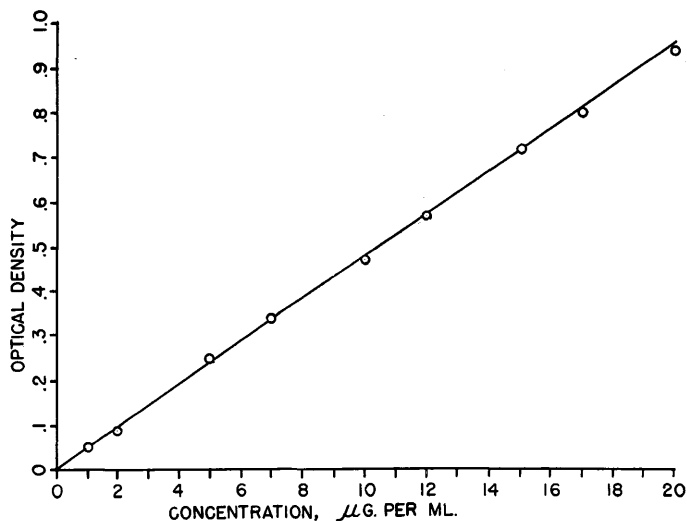


FIGURE 1. Relation of optical density to concentration of aureomycin solutions at 367 $\text{m}\mu$.

During the irradiation the sample cup was placed 2 to 3 mm below the beryllium window of a Machlett AEG-50-T x-ray tube (Machlett Laboratories, Inc., Springfield, Connecticut). The tube was operated at 35 kv and 10 ma. Data concerning the characteristics of this tube as given by Rogers (1947) indicate that the irradiation should have had a wave-length range of 0.4 to 4.4 \AA with a peak at approximately 1.5 \AA and an intensity at the sample of approximately 250,000 r/min.

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Irradiation periods of up to 15 minutes for the solutions and 20 minutes for the frozen solutions were used. A single experiment was made on dry crystals with a 15-minute irradiation.

Bioassays to determine the strength of irradiated samples and controls were made by the serial dilution method of Dornbush and Pelcak (1948) using *Bacillus cereus* No.5 as the test organism.

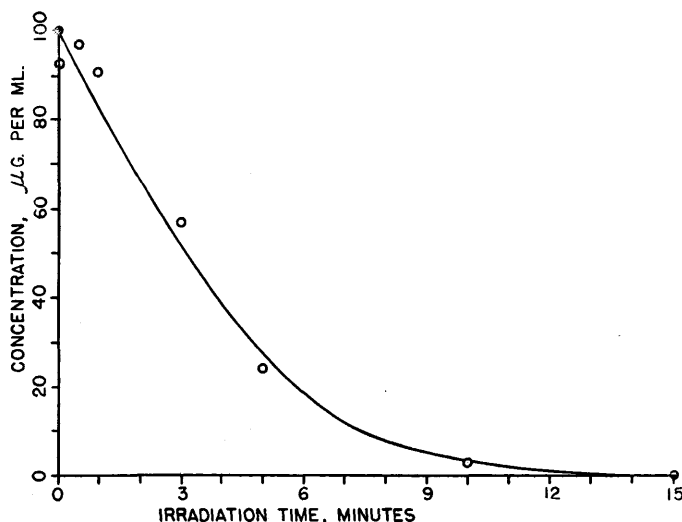


FIGURE 2. Concentration of x-rayed aureomycin solutions as determined by bioassays. Initial concentration before irradiation was 100 $\mu\text{g/ml}$ for all solutions.

The optical density of irradiated and control solutions was measured with a Beckman Model DU Spectrophotometer at a number of wavelengths in the ultra-violet. As a reference for concentration changes, measurements of optical density of various dilutions of a 20 $\mu\text{g/ml}$ solution of aureomycin were made at 367 $\text{m}\mu$ (fig. 1). The working range of the Beckman necessitated dilution of the 100 $\mu\text{g per ml}$ standards to 10 $\mu\text{g per ml}$. An attempt was made to dilute irradiated samples to 10 $\mu\text{g per ml}$ before determining optical density. This was done by assuming that they had deteriorated to the values indicated by the bioassays.

TABLE 1

Effect of soft x-rays on frozen aqueous solutions of aureomycin

Number of experiments	Irradiation time in minutes	Final concentration, average value in $\mu\text{g/ml}$
1	0	100
1	1	95
1	3	95
2	5	94
3	10	88
1	15	120
1	20	100

Initial concentration of all solutions, 100 $\mu\text{g/ml}$

RESULTS AND DISCUSSION

Biological activity of the aureomycin solutions decreased markedly with increasing irradiation time. At 3-4 minutes it had fallen to about 50 per cent of its initial value of 100 μg per ml (fig. 2). On the other hand, there was no measured activity decrease in the crystalline aureomycin and no clearcut decrease in the frozen solutions (table 1). Radiation sensitivity of substances in dilute aqueous solution and relative insensitivity as a crystalline solid or in frozen aqueous solution has been noted before. A discussion of work on these phenomena is given by O'Meara (1952).

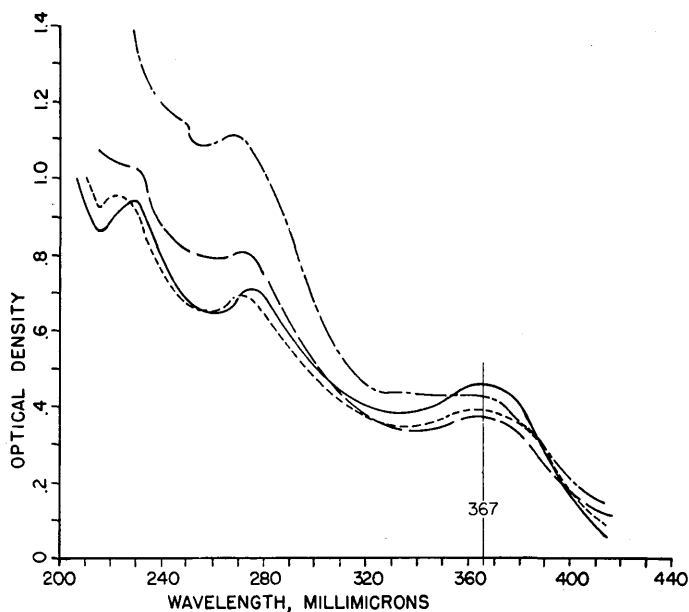


FIGURE 3. Ultraviolet absorption curves of aureomycin solutions showing comparison of control non-irradiated solution with irradiated samples. All solutions at 100 $\mu\text{g}/\text{ml}$ before irradiation.

————— Control solution, diluted 1 : 10;
 - - - - - Irradiated 1 min, diluted 1 : 10;
 ———— Irradiated 3 min, diluted 1 : 8;
 - - - - - Irradiated 5 min, diluted 1 : 6.

Curves prepared from the ultraviolet data are shown in figure 3. Interpolation of optical density values of irradiated samples at 367 $\text{m}\mu$ in figure 1 yields concentrations in μg per ml of 98, 83, 65, and 55 respectively for control, 1-minute, 3-minute, and 5-minute irradiations as compared with 100, 91, 57, and 24 for the bioassays.

Immediately after irradiation there was a noticeable darkening of the yellow color of treated solutions. This was distinguishable, when compared with controls for irradiation times as short as $\frac{1}{2}$ minute but was especially marked for longer irradiation times. Solutions continued to darken for hours after being exposed to the x-rays.

The pH of a solution before a 5-minute irradiation was 3.4. The same value was obtained on the day following irradiation for it and a non-irradiated control solution.

SUMMARY

Aqueous solutions, frozen solutions, and dry crystalline Aureomycin hydrochloride (Lederle) were subjected to doses of soft x-rays at about 250,000 r units per minute. A bioassay was then used to determine whether there had been any change in the inhibiting action of the treated material on *Bacillus cereus* No. 5. The solution (100 μ g per ml initially) lost half its strength after 3 to 4 minutes irradiation. Deterioration was confirmed with UV curves. There was no measurable change in the dry crystals after 15 minutes irradiation or in the frozen solutions after 20 minutes irradiation.

LITERATURE CITED

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